1. The scientific abstract.

There has been increasing interest in recent years in developing immunologic approaches to malignancies, and there is good evidence that the growth of renal cell carcinoma (RCC), melanoma, non-small cell lung cancer (NSCLC), and other malignancies can be modulated by the host's immune system. A recently described strategy involves the use of a vaccine in which a universal MHC-negative GM-CSF-producing "bystander cell" is mixed with irradiated but otherwise unmodified fresh autologous tumor cells (antigen source). This vaccine, called K562 Bystander GVAX®, is being developed by the Johns Hopkins Oncology Center in collaboration with Cell Genesys, Inc., and is currently being tested in Phase I/II clinical trials in patients with multiple myeloma and NSCLC (http://www.cellgenesys.com/products-cancer-vaccines.shtml).

Our laboratory has created a bystander cell line (GM.CD40L) which secretes GM-CSF and expresses CD40L on its cell surface. The rationale is that, in the context of autologous tumor cell-based vaccine formulations, these bystander cells will help recruit professional antigen presenting cells (APCs) in the form of dendritic cells (DCs) by secreting GM-CSF in the vaccine site microenvironment. The DCs will then be activated by expression of CD40L on the universal bystander cells. DCs will take up apoptotic bodies from the irradiated autologous tumor cells and
in turn will present tumor antigens in the context of MHC class I and class II. Tumor-specific DCs will then migrate to the regional lymph nodes, where T cell activation can occur, ultimately leading to systemic tumor cell killing. Mouse experiments with a murine equivalent GM-CSF-secreting bystander have supported this hypothesis, with cure of mice after vaccination with a mixture of autologous tumor and bystander cell.

This protocol describes a phase I study for cancer patients with stage IV disease who will undergo resection of symptomatic primary tumors or metastases. Tumors will be disaggregated into single cell suspensions, irradiated, frozen in aliquots of $5x10^6$ cells, and stored in liquid nitrogen until ready for use. Patients will receive three intradermal vaccine injections at 28-day intervals. On the day of vaccination, a vial of irradiated autologous tumor cells will be rapidly thawed, diluted in 10 mL sterile saline for 15-30 min, centrifuged, resuspended in 0.5 mL sterile saline, and mixed with a similarly thawed and
resuspended aliquot of irradiated GM.CD40L cells. This vaccine formulation (autologous tumor cells plus GM.CD40L bystander

cells) will then be administered to the patient. Patients will be monitored for evidence of toxicity, the development of a specific immune response, and objective tumor responses. The maximum tolerated dose (MTD) will be determined by escalating the dose of the bystander in 3 cohorts of patients.